Applied Soil Ecology 142 (2019) 59–63

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Soil solarization simulator☆

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ARTICLE INFO

Keywords:
Fusarium
Firmicutes
Real-time PCR
Simulation
Solarization

ABSTRACT

Soil solarization is an environmentally friendly physical control method used in plant protection practices. Temperature, which is the primary factor in soil solarization, can be easily adjusted with a variety of devices that can maintain constant temperature in laboratory studies. However, the temperature changes every minute and day during soil solarization in field environments.

Therefore, the objective of this work was to develop a device that simulates soil solarization in a laboratory environment in regard to temperature. For this purpose, a device was developed to reproduce temperature variations in a laboratory setting based on temperature data taken during soil solarization in a field setting. The temperature during solarization and the temperature of the simulation were compared. To demonstrate the success of the simulation, population fluctuations of two groups of microorganisms (*Firmicutes* as a bacterial phylum and *Fusarium oxysporum* as a fungal species) in the solarization and simulation settings were measured by real-time PCR and compared.

The developed system was found to simulate temperature perfectly. The mean of the difference between solarization and simulation temperature values was $-0.015\,^{\circ}C$ and $R^2 = 0.99966$ ($n = 9600$). Microorganism population changes in the two groups verified the success of the simulation. The initial population of the *Firmicutes* was 5.77×10^7 fg g^{-1} soil. This value increased to 1.16×10^8 and 1.25×10^8 fg g^{-1} soil at the end of solarization and simulation, respectively. However, population of *Fusarium oxysporum* was decreased at the end of both applications. These changes between solarization and simulation were not statistically different.

By developing this simulation, it is possible for researchers to perform preliminary studies in the laboratory many times before solarization. It is also possible to operate the simulation with previously collected temperature data for any location, year or soil depth. Source codes and schematics are available at https://github.com/uozyilmaz/SolarSim web page and are free to use.

1. Introduction

Plant protection practices involve several methods for the control of diseases, pests, and weeds in agriculture (Agrios, 2005). Chemical control methods are commonly used due to their quick response and affordable application.

Today, many regulations are being made to limit the use of pesticides in agriculture, and pressure is being applied for the withdrawal of some chemicals from the market. Researchers tend to study alternative methods (Pal and Mc Spadden Gardener, 2006). For example, soil solarization was found to be a suitable and successful alternative to methyl bromide, which is a chemical prohibited globally and in Aydin, Turkey due to adverse environmental effects (Benlioglu et al., 2002, 2001).

Soil solarization as a physical plant protection method is a technique applied before planting that is used for the control of soil-borne pathogens and weeds. This method is implemented by covering the soil surface with a transparent polyethylene cover in hot seasons, based on the principle of trapping solar radiation under the cover and killing biotic factors with increasing temperature (Katan, 1981; Katan et al., 1987; Katan and DeVay, 1991).

This method, used with suitable plants and in climatically appropriate regions, is environmentally friendly and chemical-free. Because of heat transfer, the soil should be of adequate field capacity. After spreading the transparent plastic cover, the edges should be sealed well with soil to prevent moisture loss. After a waiting period of approximately 5–6 weeks, the cover should be opened, and the planting process should immediately be started without disturbing the soil shape.

Some factors restrict researchers from working with and conducting research on this method. The most important of these restrictions is that

Abbreviations: IDE, integrated development environment; SD, Secure Digital

☆ This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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https://doi.org/10.1016/j.apsoil.2019.05.018
Received 13 August 2018; Received in revised form 2 May 2019; Accepted 23 May 2019
Available online 04 June 2019
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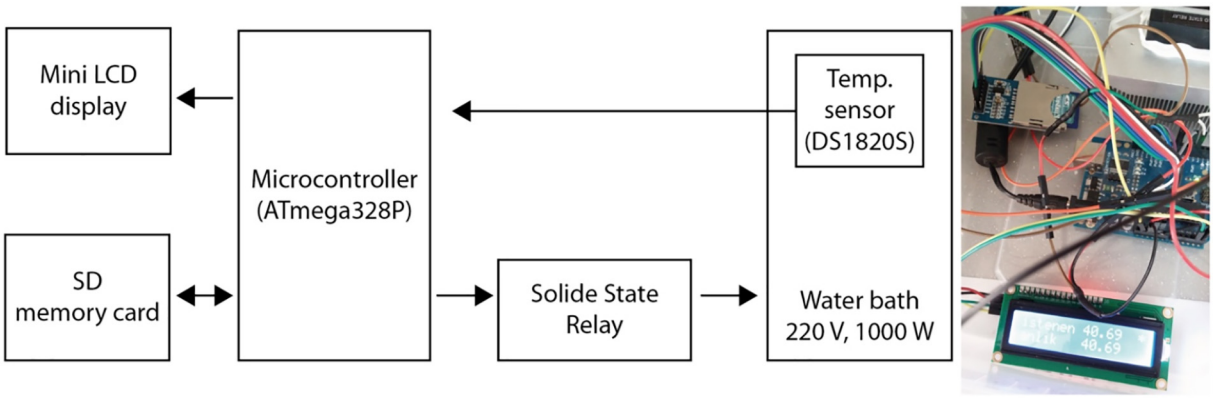

Fig. 1. SolarSim hardware diagram.

this method can only be applied once a year in the field. Therefore, it is difficult for researchers to try different things during this limited period, and they have to wait until the next year to continue their studies. Additionally, temperature differences between years may also make it difficult to interpret the results.

Before conducting field studies, preliminary experiments in the laboratory will facilitate the work of researchers. Temperature, which is the primary effect of soil solarization, can be obtained easily with many types of equipment, such as an incubator, oven, or water bath, under laboratory conditions. However, during the 40-day solarization in natural settings, the temperature changes almost every hour, and there are broad differences between day and night temperatures; further, sometimes unexpected natural events cause sudden temperature changes. It appears to be incorrect to expect the constant temperature provided in laboratory studies to represent this temperature fluctuation in nature.

Klein et al. (2007) evaluated the effects of organic additives on solarization in their study by controlling temperature via a heater unit in the laboratory with a commercial device (21× Micrologger, Campbell Scientific Inc., Logan, UT). Organic amendment application was found to be highly effective on *Fusarium oxysporum* f. sp. *radicis-lycopersici*. They ran the heating system for 28 days and, in many cases, found that it behaves the same as solarization.

Similar to this study, Gilardi et al. (2014) studied the effect of organic amendments on solarization against Fusarium wilt by simulated soil solarization and found them to be successful. They carried out their studies in a growth chamber by adjusting the temperature at intervals during the day: 6 h at 52–55 °C, 8 h at 48–50 °C and 10 h at 45–47 °C.

Schönfeld et al. (2003) found that the addition of compost in simulated solarization reduced the *Ralstonia solanacearum* population. The simulation was done in a growth chamber by filling soil in polyvinyl chloride rings (19.2 cm diameter and 5 cm height).

Yildiz et al. (2010) investigated the effects of different types of plastic coverings used in solarization on *Macrophomina phaseolina* and weeds. During their research, they carried out tests on *M. phaseolina*'s temperature-survival relationship considering different incubation temperatures and times provided by a water bath set to a constant temperature.

In previous studies, either a limited range of temperature or fixed temperatures were studied. However, during solarization in nature, temperatures vary from throughout the duration of solarization.

The objective of this work was to develop a device that simulates soil solarization in laboratory environment in terms of temperature. Simulation allows researchers to perform preliminary experiments in the laboratory many times before solarization. It is also possible to operate simulation with previously collected temperature data from any location, year or soil depth.

2. Materials and methods

2.1. Solarization and soil sampling

The raw soil temperature data and soil samples used in the development of the solarization simulator device (SolarSim) was obtained during solarization of a field (37°52′45.9″N 28°09′20.3″E) before strawberry (*Fragaria* × *ananassa*) production in the Sultanhisar district of Aydin province in Turkey. The soil was a sandy loam (Typic Xerofluvent) (Altınbaş et al., 1999; Soil Survey Staff, 2010).

The experiment had three replications of 250 square meters each. During the soil solarization performed for 40 days between 27 June–6 August 2014, soil temperatures of 5, 10 and 20 cm depth were stored in a data logger (Onset Hobo data logger, TMC6-HD temperature sensor). Immediately before and after soil solarization, 100 g soil samples were taken at 20 cm depth from ten different randomly chosen points for each replication. The soil of each replication was mixed, sieved through a 0.5-mm sieve and stored at −80 °C during the solarization period.

2.2. Simulation device

SolarSim consists of two parts: hardware (Fig. 1) and software. The temperature data collected from the field during soil solarization was first prepared with computer software (developed in this study, codes not shown. Integrated development environment [IDE] and compiler: Xcode 7.0 by Apple). This computer program processed the temperature data that was obtained at 1-hour intervals and produced nine additional temperature values between two consecutive temperature measurements by increasing or decreasing the values in a linear fashion. This approach was used to soften the transition between the two hourly temperature measurements. After preparing the data, the same program compressed the data and prepared it for SolarSim. This data file was copied onto an SD (Secure Digital) card and installed to SolarSim.

The operating logic of SolarSim processes the given temperature list and adjusts the temperature of the water bath. The system is designed to adjust the temperature in a cyclical pattern of heating-waiting-heating (in pulses), because the temperature fluctuations are too high in the conventional thermostat working principle. According to this design, the device adjusts the temperature by calculating the heating and waiting duration (1) and works similar to a heartbeat. The device also saves current temperature in the water bath on the SD card (IDE and compiler: Arduino 1.6.5). At the end of the simulation, the temperature data recorded on the SD card is transferred to the computer for evaluation. SolarSim, running at room temperature, works independently of the computer.

$$run_time = ((\text{maxrun} - \text{minrun})/\text{maxdif}) \times (\text{set} - \text{cur})$$
$$delay_time = ((\text{maxdelay} - \text{mindelay})/\text{maxdif}) \times (\text{maxdif} - (\text{set} - \text{cur})) \quad (1)$$

set: target temperature in Celsius
cur: current temperature in Celsius
minrun: minimum run time, default 3000 ms
maxrun: maximum run time, default 150 ms
mindelay: minimum delay time, default 100 ms
maxdelay: maximum delay time, default 8000 ms
maxdif: threshold of switching continuous to pulse run, default 20 °C.

2.3. Comparison of simulation and solarization

The success of the device has been checked in two stages. In the first stage, the difference between the temperature data recorded in the water bath during the simulation and the temperature values required to adjust the device were compared. Additionally, the speed of the temperature increases and decreases in nature was used to evaluate whether the device was adequate. In the second stage, in solarization/simulation and before/after, population changes of some microorganism groups in the soil were measured.

A sample of 2 g of soil was taken before soil solarization and placed in a screw capped thin wall glass test tube in SolarSim; three replicates were performed. The device was run. At the end of the operation, total DNA was extracted from soil samples before solarization, after solarization, and after simulation by using a commercial DNA extraction kit (MOBIO PowerSoil) according to the kit instructions.

Population changes of *Firmicutes* and *Fusarium oxysporum* in both the simulation and solarization soil samples were calculated by using real-time polymerase chain reaction (real-time PCR). Real-time PCR (BioRad CFX96) was performed under the following conditions: initial denaturation for 3 min at 98 °C, followed by 45 cycles of denaturation at 95 °C for 10 s, annealing at 63 °C (for *Firmicutes*) or 61 °C (for *Fusarium*) for 5 s, and elongation at 72 °C for 30 s (Ozyilmaz et al., 2016). For *Firmicutes* Lgc353/Eub518 (Forward: 5′-GCA GTA GGG AAT CTT CCG-3′, Reverse: 5′-ATT ACC GCG GCT GCT GG-3′) (Fierer et al., 2005) and for *Fusarium oxysporum* FOF1/FOR1 (Forward: 5′-ACA TAC CAC TTG TTG CCT CG-3′, Reverse: 5′-CGC CAA TCA ATT TGA GGA ACG-3′) (Mishra et al., 2003) primer pairs were used.

To determine the amount of microorganism groups per gram of soil, standard curve formulas were calculated using *Firmicutes* strain C5 (Adnan Menderes University, Faculty of Agriculture, Department of Plant Protection) and *Fusarium oxysporum* strain Forl-S (Ege University, Faculty of Agriculture, Department of Plant Protection) (data not shown). After solarization and simulation, the mean amounts were compared within each microorganism group.

2.4. Statistical analysis

Population changes of two microorganism groups in the solarization and simulation were analyzed using Student's *t*-test. The difference between the solarization and simulation temperature data was evaluated using matched pairs analysis (JMP® Ver. 12.0, SAS Inst. Inc., Cary, NC).

3. Results and discussion

Solarization, which is widely used in strawberry production in the Sultanhisar district of Aydin province, is applied at the end of June through early July. In Turkey, according to average daily temperatures between 1970 and 2017, the hottest days occur in July–August. In these months, the west and south coasts of the country and the southeastern parts are warmer than the central and north parts (Anonymous, 2018). These months are very suitable for solarization. During this study, the solarization was started at the end of June and continued for 40 days. The average daily soil temperatures were measured at different depths during solarization, as shown in Fig. 2. The graph shows that the average daily temperature increased and fluctuated day by day. In addition, temperatures near the surface were higher, and the average temperature decreased with depth.

Even at twenty centimeters depth, it was found that the average daily maximum temperatures reached 44 °C, and the average daily temperatures reached 40 °C. The maximum daily temperatures at different depths ranged from 44 to 55 °C (Table 1). It was observed that the hours of highest average daily temperature at five centimeters depth was between 14:00–20:00 with a peak at 16:00. With increasing depth, high temperatures hours shifted forward (Fig. 2, Table 1). Strawberry plant roots are usually found in the first 15–30 cm (Ashton, 2008; Poling, 1993). For this reason, raw temperature data and soil samples were evaluated by taking into consideration the depth of 20 cm. Similarly, Klein et al. (2007) evaluated their studies using data obtained at a depth of 20 cm.

During solarization, soil temperatures were recorded at 1-hour intervals. Computer software produced nine more temperature values between each hour to change the temperature values gradually and prevent sudden temperature changes. As a result, approximately 9600 temperature values were created in a series to adjust SolarSim and were copied to the device.

The device was operated with this temperature series. The soil samples taken before solarization were placed in test tubes in SolarSim, and the temperature of the water bath was recorded during the simulation. At the end of forty days, the temperature data was transferred to the computer for analysis. In Fig. 3 (left), the data obtained at the end of the simulation shows a randomly selected 10-hour period. As the graph shows, there is harmony between the expected (solarization) and actual (simulation) values.

The mean of the difference between expected and recorded temperature values was −0.015 °C, and the maximum deviation in the negative direction was −0.27 °C and in the positive direction was 0.18 °C (Fig. 3 [right], matched pairs analysis $R^2 = 0.99966$).

During solarization, at 20 cm depth, the fastest temperature decrease was 0.96 °C h^{-1} on 7/8/2014 at 23:00, and the fastest temperature increase was 1.41 °C h^{-1} on 7/30/2014 at 16:00. The simulation device reached the desired temperature within a couple of minutes, and it can quickly reach the sudden temperature increases observed in nature. When SolarSim, which operates at 40 °C, stopped heating, it was observed to cool at a rate of 13 °C h^{-1}, which is faster than solarization, and did not need a cooling system.

At the end of solarization and simulation, real-time PCR experiments were conducted to determine the population changes of *Firmicutes* and *Fusarium oxysporum*. Standard curve equation efficiency was found to be 91% and 103%, respectively, and was within reasonable limits (Taylor et al., 2010) for quantification calculations. The change in DNA amounts of microorganisms after solarization and simulation is given in Table 2.

Firmicutes is a phylum that contains gram-positive bacteria in abundance in the soil and can easily resist increased temperature through their endospores. Table 2 shows that *Firmicutes* were increased, as expected, both at the end of solarization and simulation. In addition, there was no statistically significant difference between soil solarization and simulation results. A similar population increase of *Firmicutes* was found at the end of soil solarization (Ozyilmaz et al., 2016). *Fusarium oxysporum* species, which contain plant pathogens, generally have low heat tolerance and are easily affected by solarization (Barakat and AL-Masri, 2012; Ozyilmaz et al., 2016). In this study, the population of *Fusarium oxysporum* was decreased both after solarization and simulation, and there was no significant difference between solarization and simulation.

Researchers usually evaluate the effects of temperature on microorganisms with laboratory equipment that is set to a constant temperature and does not reflect natural variations. In this study, temperature fluctuations in soil solarization were successfully simulated in a laboratory environment. The device called SolarSim (Source codes and schematics are available at https://github.com/uozyilmaz/

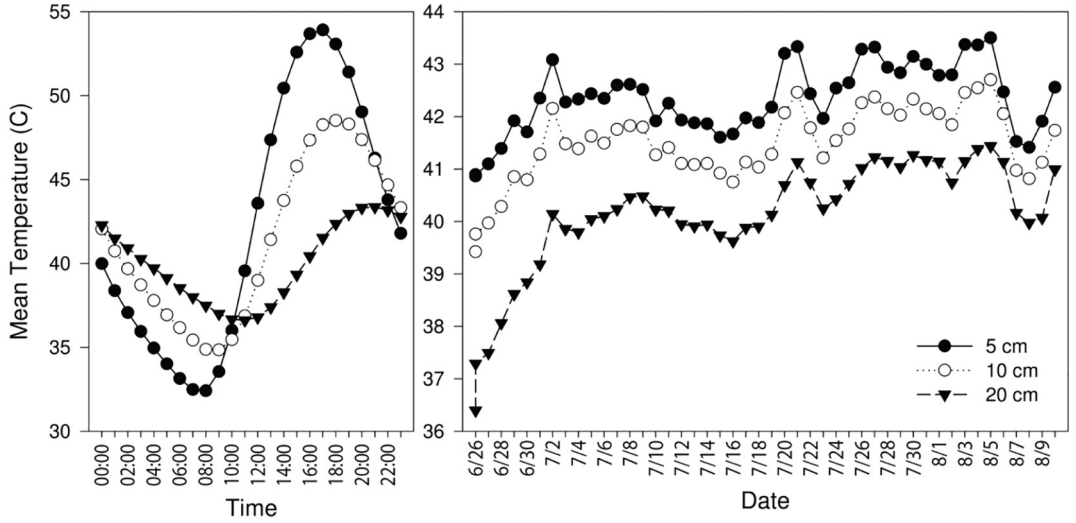

Fig. 2. Hourly (left) and daily (right) average temperatures taken from different depths during solarization.

Table 1
The average of temperatures recorded from different depths during solarization.

Depths	Daily temperature mean[a]			Hottest hours[b]
	Minimum	Maximum	Average	
	˚C	˚C	˚C	
5 cm	30.31 ± 0.81	55.35 ± 0.91	42.42 ± 0.66	16:00–17:00
10 cm	31.93 ± 1.06	49.56 ± 0.60	41.54 ± 0.69	17:00–19:00
20 cm	33.59 ± 1.17	44.40 ± 0.82	40.16 ± 0.99	19:00–22:00

[a] Mean and standard deviation were obtained from 40-day temperature data.
[b] The hottest time range of the day according to daily average temperatures.

Table 2
The change in DNA amounts of microorganisms after solarization and simulation.

Microorganism/ treatment	Sampling time			
	Before treatment		After treatment	
	Cycle threshold[a]	DNA amount	Cycle threshold[a]	DNA amount[b]
	Ct	fg g^{-1} soil	Ct	fg g^{-1} soil
Firmicutes				
Solarization	26.82	5.77×10^7	25.74	1.16×10^8
Simulation	nt[c]	nt[c]	25.61	1.25×10^8
F. oxysporum				
Solarization	35.57	1837	42.57	17
Simulation	nt[c]	nt[c]	41.08	50

[a] Real-time PCR cycle threshold, all values were the average of three replicates.
[b] Two microorganism groups were evaluated separately, and there was no statistically significant difference between solarization and simulation values (Student's *t*-test, $p > 0.01$).
[c] Not tested, soil samples were the same before the applications.

SolarSim for free use) can support laboratory studies in terms of temperature control. The population changes of the *Firmicutes* and *Fusarium oxysporum* microorganism groups in the soil samples that were simulated were statistically similar to the results of soil solarization according to the molecular tests. Perhaps the most impressive feature of the developed device is that it mimics solarization temperature in the laboratory with a whole temperature value series. The temperature data used is not representative or an average of a limited time series. The device will allow researchers to perform pretests that reflect true temperature variations in the laboratory before solarization. The effects of solarization on soil chemical, physical or biological structure can easily be evaluated with preliminary studies before field trials. In addition, the device can be run with different location, year, season, and soil depth data series. In some cases, trials must be repeated under the same conditions. In different years, repeated solarization applications may

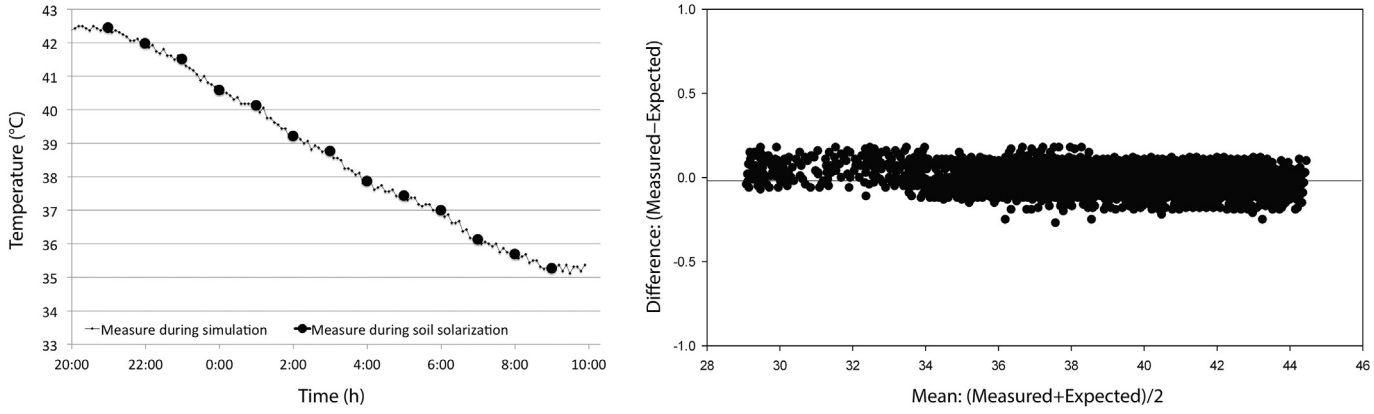

Fig. 3. Randomly selected 10-hour period's temperature data during the simulation (left). The mean of the difference between expected (solarization) and recorded (simulation) temperature values (right).

generate nonstable data and make the results difficult to evaluate due to the seasonal differences between years. SolarSim can help by reproducing the same conditions over and over again. This device simulates solarization only in terms of the temperature responsible for the main effect of soil solarization. Soil moisture is an essential factor in solarization for the conduction of heat. However, properly applied solarization maintains the necessary moisture. In addition to this study, moisture, oxygen, carbon dioxide and other possible factors that affect soil solarization must be studied.

Declaration of Competing Interest

None.

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